



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| (51) International Patent Classification ⁵ : C12M 1/12, C12Q 1/06, C12M 1/34, 1/36, 1/38 | A1 | (11) International Publication Number: WO 94/21780 (43) International Publication Date: 29 September 1994 (29.09.94) |
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(21) International Application Number: PCT/CA94/00101

(22) International Filing Date: 25 February 1994 (25.02.94)

(30) Priority Data:
033,228 18 March 1993 (18.03.93) US

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(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

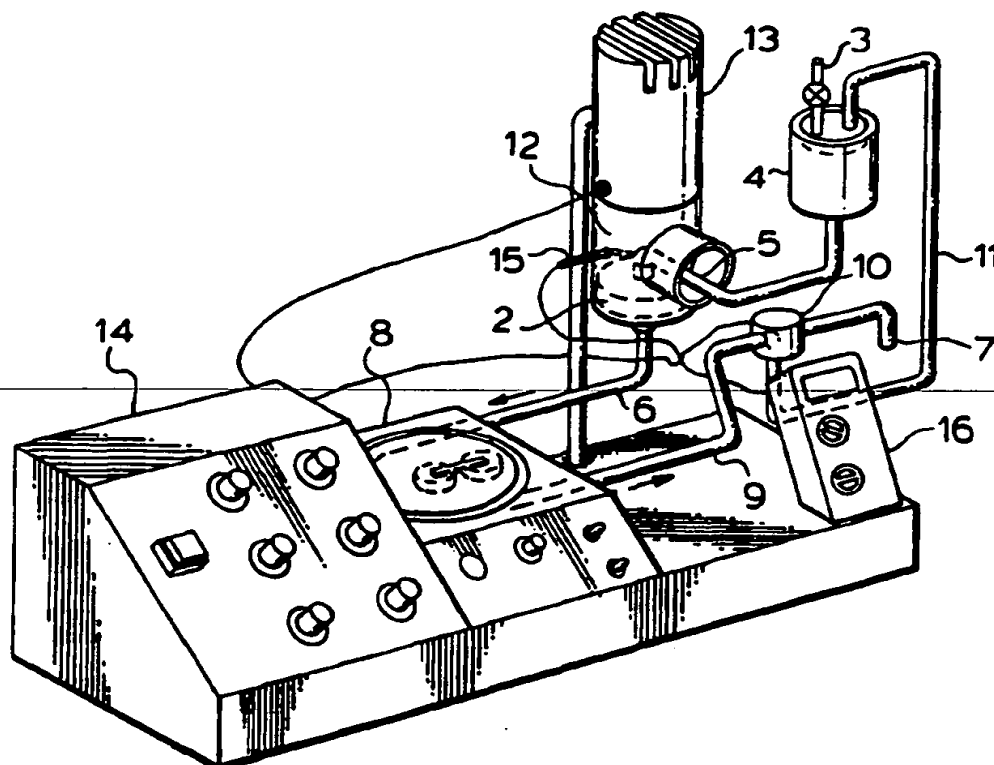
Published

With international search report.

(54) Title: APPARATUS FOR THE QUANTITATIVE DETERMINATION OF PARTICULATE ANALYTES

(57) Abstract

An apparatus is provided for detecting and enumerating a particulate analyte present in a liquid sample comprising a filter element (1), a holder (2) to support the filter element (1), an inlet duct (3) to deliver the liquid sample to the filter element (1), an outlet duct (6) to receive filtrate from the filter element (1), a heat transfer means (13) to transfer heat to and from the filter element (1), a controller (14) operable upon the heat transfer means to maintain the filter element (1) at preselected temperatures for preselected time periods, a flow controller (10) operable in a first position to connect the inlet and outlet ducts (3, 6) for recirculation of fluid through the filter element (1) and in a second position to prevent recirculation of fluid through the filter element (1) and a pump (8) for recirculating fluid through the filter element (1). A method is also provided for detecting and enumerating a particulate analyte in a liquid sample, utilising the apparatus of the invention.



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APPARATUS FOR THE QUANTITATIVE DETERMINATION OF
PARTICULATE ANALYTES

5 The invention relates to an apparatus for the
quantitative determination of particulate analytes,
including microorganisms.

Background of the Invention

10 It is frequently desirable to quantitate
microorganisms present in soil samples, water, food
samples and samples of biological materials of medical or
veterinary interest. Traditionally, quantitative
estimation of microorganisms in medical or environmental
15 samples have been based upon colony counts after
culturing appropriately diluted samples on nutrient agar
plates.

 Species and strains of microorganisms in such
samples can be detected and identified by probing for
20 specific DNA sequences, as described in U.S. Patent No.
4358535 to Falkow et al. This method, however, is not
usually quantitative.

 Alternatively, specific DNA sequences may be
detected after selective amplification using the
25 polymerase chain reaction (PCP), as described by Mullis,
K. B. et al., (1987), Methods in Enzymol., vol. 155, pp.
335 - 351 and Saiki, R. K. et al., (1988), Science, vol.
239, pp. 487 - 494.

 This process involves repeated cycles of incubation
30 of the sample with an appropriate reagent, which includes
a DNA polymerase enzyme and DNA synthesis
oligonucleotides as primers. The initial step in each
cycle thermally denatures the DNA. This is followed by
annealing and new DNA synthesis steps at lower
35 temperatures.

Samples for analysis by selective amplification by PCR are usually contained in micro-test tubes during the necessary manipulations.

5 The test tubes can be subjected to the necessary time/temperature cycles in apparatus such as the Perkin-Elmer/Cetus DNA Thermal Cyclor which comprises a heating block with apertures to hold the tubes and appropriate controls to maintain the block and the tubes at the desired temperatures for the desired time periods.

10 The results obtainable by this method are, at most, semi-quantitative. For example, the reaction mixture resulting from PCR amplification may be analysed by agarose gel electrophoresis, the intensity of the resulting bands providing only a very rough quantitation
15 of the original target DNA sequence.

There remains a need for a simple convenient method which will allow direct quantitation of the microorganisms of interest without the need for culture and for an apparatus which will facilitate the carrying
20 out of such a method.

Summary of Invention

in accordance with one embodiment, the invention provides an apparatus for detecting and enumerating a
25 particulate analyte present in a liquid sample comprising a filter element, a holder to support said filter element, an inlet duct to deliver said liquid sample to said filter element, an outlet duct to receive filtrate from said filter element, a heat transfer means to
30 transfer heat to and from said filter element, a controller operable upon said heat transfer means to maintain said filter element at preselected temperatures for preselected time periods, a flow controller operable in a first position to connect said inlet and outlet
35 ducts for recirculation of fluid through said filter element and in a second position to prevent recirculation

of fluid through said filter element and a pump for recirculating fluid through said filter element. In accordance with a further embodiment, an tit apparatus is provided for detecting and enumerating a
5 particulate analyte present in a liquid sample comprising at least one filter for retaining the particulate analyte, a holder for supporting said filter, temperature control means for adjusting the filter to a selected temperature, a first conduit for delivering liquids to
10 said filter and a second conduit for removing filtrates from said filter, flow control means whereby said first and second conduits may be connected directly or indirectly to form a closed loop for recirculation of fluid through the filter, a controller for controlling
15 operation of said temperature control means and a pump for recirculation of fluid through the filter.

In accordance with a further embodiment, a method is provided for detecting and enumerating a particulate analyte present in a liquid sample comprising delivering
20 said liquid sample through an inlet duct to a filter element, removing filtrate flowing through the filter element through an outlet duct while retaining the particulate analyte on the filter element, delivering a suitable reagent to said filter element while maintaining
25 the filter element at a suitable temperature to fix a selected component of said particulate analyte to said filter element, and delivering to the filter element suitable detection reagents to render the selected
component detectable, whereupon, on delivery of said
30 reagents, said inlet duct is connected directly or indirectly to said outlet duct to permit recirculation of said reagents through said filter element, and said filter element is subjected for a selected number of repetitions to a selected temperature cycle by operation
35 of a heat transfer means operable to transfer heat to and from said filter element, said reagents being recirculated through said filter element for selected

time periods during said temperature cycle by operation of a pump, operation of said heat transfer means and said pump being controlled by a controller.

5 Brief Description of the Drawings

Certain embodiments of the invention are described, reference being made to the accompanying drawings, wherein:

10 Figure 1 shows a schematic representation of one embodiment of the invention.

Figure 2 shows greater detail of a portion of the apparatus of Figure 1.

15 Figure 3 shows a schematic representation of fluid flow through the apparatus of the invention in closed configuration.

Figure 4 shows a schematic representation of fluid flow through the apparatus of the invention when in open configuration.

20

Detailed Description of the Invention

The present invention provides an apparatus which facilitates the quantitative determination of a
25 particulate analyte in a sample.

The particulate analyte is trapped as discrete particles on the filter of the apparatus and fixed to the filter by a suitable method. By means of the apparatus, the particulate analyte is then exposed to various

30 reagents and reaction conditions selected to render the particulate analyte of interest detectable and countable.

Alternatively, where the particulate analyte is to be rendered detectable and countable by a multi-step reaction process, one or more of the reaction steps may
35 be carried out by means of the apparatus of the invention, with an additional step or steps being carried out on the filter after its removal from the apparatus.

As will be understood by those skilled in the art, a sample dilution is employed which will result in the trapping of the particulate analyte on the filter as discrete particles sufficiently spaced from each other to be counted. An appropriate dilution can be determined empirically.

Any particulate analyte which can be trapped on and fixed to the filter and rendered detectable and countable thereon by exposure to suitable reagents may be quantitated using the apparatus of the invention.

The apparatus is particularly useful for the determination of microorganisms, which term is used to include any unicellular organisms such as bacteria, fungi, protozoa and single cell preparations of eukaryotic cells.

Microorganism-containing samples may include water samples, soil samples, biological samples such as body fluids or tissue samples and food or food-processing samples. A suitable aqueous suspension is prepared and filtered through the apparatus of invention, as will be understood by those skilled in the art.

Figures 1 to 4 illustrate one embodiment of the apparatus of the invention. A filter 1 of appropriate porosity is supported in a holder 2. Reagent or sample solutions are introduced into the apparatus through inlet port 3 and reservoir 4 to inlet duct 5 which delivers solution to filter 1.

Solutions are pumped through the filter by operation of peristaltic pump 8. Filtrates passing through filter 1 are conveyed by outlet duct 6 to waste at outlet 7 when the apparatus is operated as an open system, as in Figure 4.

Alternatively, as seen in Figure 3, a closed system can be formed by adjustment of flow controller 10 to direct liquid flow through return line 11 back to reservoir 4, so that once the system is charged with a desired reagent, this may be pumped continuously through

filter 1 and around the closed loop 9 for a desired time interval by means of pump 8.

The filter holder 2 is supported in filter housing 12. Alternatively, filter holder 2 and housing 12 may be of integral construction. Heat gun 13 is positioned adjacent to filter 1 and can be operated to direct a stream of hot or cool air over filter 1 so as to maintain filter 1 at preselected temperatures. A commercially available hand held-type heat gun has been found to be satisfactory.

Other heat transfer means may be employed to transfer heat to and from the filter, including use of heating and cooling coils, for example located in filter holder 2, and use of a liquid as a heat transfer medium. Operation of heat gun 13 is controlled by controller 14. The temperature of the filter 1 is monitored by an integral thermocouple 15 placed directly in the liquid flow adjacent the filter with read-out on digital thermometer 16.

Controller 14 is programmed to provide thermostatic control of the temperature of filter 1, the heat gun being activated by the controller to transfer heat to and from filter 1 so as to maintain filter 1 at a preselected temperature for a preselected period of time.

In accordance with a preferred embodiment, the controller 14 is also programmed to control operation of the pump 8. As it may be desirable for certain applications to switch off the pump when the filter is in a particular temperature range, as will be described, the pump controller is preferable thermostatically controlled.

It is generally not possible to avoid completely the occurrence of bubbles in the circulating solutions. Bubbling may be controlled by returning the recirculated solution from return duct 11 into the top of reservoir 4 as seen in Figures 3 and 4.

Other bubbling control systems will be known to those skilled in the art.

The filter 1 must be of a porosity to retain the organisms of interest. The material of the filter will
5 be selected depending on the method to be used for detection, of the particles. For example, if high temperatures are involved, the filter must be able to withstand these temperatures

The filter holder 2, filter housing 12 and tubing of
10 the circulation loop 9 must also be of materials compatible with the reagents and temperatures of the reactions to be employed. For reactions involving high temperatures, a nylon filter holder and housing and teflon tubing have been found to be satisfactory. A
15 filter holder with minimal void volume and thin walls is preferred to facilitate rapid temperature change of both holder 2 and filter 1.

Filter holder 2 was machined from a solid block of nylon and had an outer diameter of 40 mm, wall thickness
20 of 2 mm and a void volume of approximately 3.0 mL.

In accordance with a further embodiment, two or more filters supported in filter holders may be arranged in parallel, being supplied with reagents from a common reservoir by branches of inlet line 5 and being drained
25 by outlet lines which converge into a common outlet line 6. Such parallel filter holders and their filters may, for example, be used for samples of different dilutions or from different sites or sources.

In accordance with a further embodiment, two or more
30 filters of differing porosity may be arranged in stacked filter holders to retain analytes of differing particle sizes. In this case, the void volume of the apparatus will be larger, and longer cycle times may be required to attain a predetermined temperature, as will be understood
35 by those skilled in the art.

To exemplify the procedures and apparatus of the invention, a method for the quantitative determination of

microorganisms by means of PCR amplification will be described. The apparatus of the invention is especially useful for this method. It should be understood, however, that the apparatus of the invention is also
5 useful for other applications, especially where the quantitative determination of a particular analyte is required.

A liquid sample containing the microorganisms to be enumerated is introduced into the apparatus through the
10 inlet port and pumped through the filter with the system in open configuration as in Figure 4, so that the microorganisms are deposited on the filter and the filtrate is pumped to waste.

The microorganisms are fixed to the filter by
15 heating the filter, by means of the heat gun, to an appropriate temperature for an appropriate time, followed by pumping of a suitable lysing reagent through the filter.

The DNA of the lysed microorganisms becomes fixed to
20 the filter, along with other components of the cell lysate, at the sites on the filter where the microorganisms were deposited. The DNA is then directly subjected to amplification by PCR at these sites.

A solution containing the necessary reagents and
25 diagnostic primers appropriate to the organisms of interest is introduced into the apparatus through the inlet port and reagent reservoir and the system is converted to a closed configuration by operation of flow controller 10. The reagent mixture is circulated as
30 required, as will be described, and the filter is adjusted to and held at the temperature required for each stage of the PCR process by operation of the heat transfer means.

Operation of the heat gun or other heat transfer
35 means is controlled by the controller 14, which is programmed to provide thermostatic control of the filter temperature, so as to maintain the filter at a suitable

temperature for a suitable period of time for each step of the polymerase chain reaction. Such temperatures and times are known to those skilled in the art. The cycle of temperature steps is repeated a sufficient number of
5 times to achieve the desired degree of DNA amplification, as will be understood by those skilled in the art. The inventors have found, using the apparatus of the invention, that about 25 to about 50 cycles of DNA synthesis are satisfactory for enumeration of
10 microorganisms.

The controller may also be programmed to control operation of the pump. As it is desirable to stop the pump when the filter is at high temperatures, as described below, the pump controller may preferably be
15 thermostatically controlled.

For DNA amplification by PCR using the apparatus of the invention, filters of appropriate size have been cut from Biotrans nylon membrane, Catalogue Number 810205, 0.45 μm pore size. Other filter materials are also
20 suitable, including teflon or nylon filters such as Millipore, Type FH-Fluoropore and Whatman, Type 7404, respectively.

A filter of fibrous texture is preferred for optimal retention of single-stranded DNA target template and of
25 newly synthesised double-stranded DNA product.

When the amplification process has been carried out for the desired number of cycles, the apparatus is emptied of reagent mixture and the filter is raised to
920 for 15 min. to denature the DNA and fix it to the
30 filter. The DNA may then be rendered visible by application of appropriate reagents to the filter, either by pumping these through the filter while it is contained in the apparatus of the invention or the filter may be removed and the reagents applied to the filter by
35 spraying or dipping.

DNA may be rendered visible by staining the filter with a DNA stain such as Hoechst 33258, DAPI or ethidium bromide and the resulting stained spots on the filter are counted either directly or under UV light. The amplified
5 DNA spots on the filter may also be directly visualised and counted under UV light.

It may be desirable to detect and count one or more subsets of organisms within the population of organisms whose DNA was selectively amplified by PCR using a single
10 pair of primers. For example, the selected primers may be diagnostic of a particular species of organism and strains of this species may be distinguished and counted on the filter by means of strain specific probes as will be understood by those skilled in the art. The probe
15 carries a suitable label, such as a radioactive label, which permits detection and enumeration of the strain of interest. Where the probe is radioactive, the filter is placed in contact with x-ray film and spots on the developed film are counted to provide enumeration of the
20 organism of interest.

Other probe labels will be known to those skilled in the art and include chromogenic compounds which can be reacted with a suitable reagent to give a detectable coloured compound.

25 Alternatively, two or more cell types or strains may be enumerated on the same filter by using a primer mixture containing a primer pair diagnostic for each cell type or strain in the amplification process, followed by
30 probing with a probe selective for each of the cell types or strains of interest. These selective probes may optionally carry labels giving rise to different coloured compounds, so that spots of different colours are produced on the filter, each colour corresponding to a cell type or strain of interest.

35 As will be understood by those skilled in the art, the apparatus of the invention can be used for detection and enumeration of particulate analytes by methods other

than PCR amplification. For example, ligase chain reactions may be carried out in the apparatus, or analytes may be rendered detectable by enzyme immunoassay techniques..

- 5 The following example further illustrates the use of the apparatus of the invention.

Example 1

Quantitative Determination of Genetically Modified
10 Rhizobium meliloti in soil

Rhizobium meliloti strain 825 is a soil inoculant strain used to enhance symbiotic nitrogen fixation of alfalfa. It contains a diagnostic DNA sequence
15 containing a novel transposition copy of the DNA insertion sequence ISRm1. This DNA locus has been sequenced and PCR primers selected and prepared for its amplification, as described in U.S. Patent Application, Serial No. 07/613,061 (Wheatcroft and Wyndham) the
20 contents of which are incorporated herein by reference).

After introduction of R. meliloti 825 into soil, a soil sample was obtained for enumeration of the organism of interest. 0.1 g soil was shaken up with 10 ml suspension buffer (50 mM Tris/HCl, pH 8.0, containing 25%
25 W/v sucrose) and after centrifugation of the suspension at 1000 rpm for 5 min to pellet soil particles, a series of dilutions ($\times 10^{-4}$, $\times 10^{-5}$, $\times 10^{-6}$) of the supernatant were made in water. The appropriate dilution for optimal
enumeration is determined by filtering and processing a
30 sample of each dilution in the series and selecting a dilution which gives good distribution of the microorganism as discrete countable spots.

For each suspension dilution analysed, 0.5 ml of the suspension was introduced into the apparatus of Figures 1
35 to 4 through the inlet port and the pump was activated to draw the suspending fluid through the filter and to waste through the outlet line. The filter was raised to 90°C

by application of heat by means of the heat gun and held at that temperature for 15 min. without pumping.

The heat gun was switched off and 0.5 ml lysing reagent (10 mM EDTA in suspension buffer with 1.5 mg/ml lysozyme added immediately before use), was introduced through inlet port and drawn through filter to waste by pumping.

The filter was heated to 92°C for 15 min without pumping and then was washed with 2 ml. water which was pumped to waste.

The apparatus was charged with 5.0 ml. reaction mixture through the inlet port. Reaction mixture contained 50 mM/KCl; 10 mM Tris/HCl, pH 9.0; 0.1% v/v Triton X-100; dATP, dCTP, dTTP and dGTP, each 0.2 mM; and (added immediately before use) 0.05 ml of each primer at 0.01 mM and 5 units of Taq DNA polymerase.

Flow controller 10 was adjusted to the closed loop position so that the reaction mixture could be recirculated through the filter and round closed loop 9 by means of the pump.

The filter was raised to 94°C and held at that temperature for 2 min, followed by 2.5 min. at 50°C and 3 min. at 74°C. Filter temperature was raised or lowered as required by blowing warm or cool air over the filter and filter holder by means of the heat gun. The circulation pump was stopped during steps carried out at about 90°C or higher to reduce loss of enzyme.

The filter was taken through the described temperature/time cycle for 30 repetitions.

After the amplification period was complete, the reaction mixture was voided and the filter raised to 92°C for 15 min. to denature the newly synthesised DNA.

The filter was removed from the apparatus and treated by probing with a radio labelled probe homologous to the diagnostic sequence of strain 825, followed by autoradiography and counting of spots where the cells of this specific strain had been lysed.

As indicated above, it was found preferable for optimal conservation of enzyme to stop the circulation pump during high temperature stages of the amplification process when using Taq DNA polymerase.

5 As will be understood by those skilled in the art, a less heat stable DNA polymerase such as Klenow DNA polymerase may also be employed for amplification. In this case, the filter is raised to 94°C for 2 min. followed by 5 min. at 42°C. When using this enzyme, the
10 circulation pump is operated only when the filter temperature is below about 45°C for optimal preservation of enzyme.

If the circulation pump is switched off during higher temperature stages, as described above, the
15 precise control of temperature in these higher ranges is likely to be less critical than with conventional PCR techniques.

The present invention is not limited to the features of the embodiments described herein, but includes all
20 variations and modifications within the scope of the claims.

We claim:

1. An apparatus for detecting and enumerating a particulate analyte present in a liquid sample comprising
5 a filter element, a holder to support said filter element, an inlet duct to deliver said liquid sample to said filter element, an outlet duct to receive filtrate from said filter element, a heat transfer means to transfer heat to and from said filter
10 element, a controller operable upon said heat transfer means to maintain said filter element at preselected temperatures for preselected time periods, a flow controller operable in a first position to connect said inlet and outlet ducts for
15 recirculation of fluid through said filter element and in a second position to prevent recirculation of fluid through said filter element and a pump for recirculating fluid through said filter element.
 - 20 2. An apparatus according to claim 1 wherein said holder includes a chamber and said filter element is located within said chamber, said inlet and outlet ducts being connected to said chamber on opposite sides of said filter.
 - 25 3. An apparatus according to claim 2 wherein said heat transfer means includes a fan operable to direct an air stream over said chamber.
-
- 30 4. An apparatus according to claim 3 wherein a heating element is located in said airstream to elevate the temperature thereof.
 5. An apparatus according to claim 4 wherein said
35 chamber and said fan are located in a supporting duct and an exhaust conduit is provided in said supporting duct between said fan and said chamber.

6. An apparatus according to claim 5 wherein said inlet duct is inserted through said exhaust conduit to connect to said chamber.
- 5 7. An apparatus according to claim 2 wherein a temperature monitor is located in said inlet duct or in said outlet duct adjacent said filter and is operably connected to said controller to regulate operation of said heat transfer means.
- 10 8. An apparatus according to claim 2 wherein said pump is located in said outlet duct.
9. An apparatus according to claim 8 wherein
15 said pump is controlled by said controller.
10. An apparatus according to claim 9 wherein said pump is a peristaltic pump.
- 20 11. An apparatus in accordance with claim 1 wherein said filter element is a nylon or teflon filter element.
12. An apparatus in accordance with claim 1
25 further comprising debubbling means connected to said outlet duct to reduce circulation of bubbles through said filter.
-
13. An apparatus in accordance with claim 2 further
30 comprising a reservoir to deliver reagents to said inlet duct and wherein said outlet duct is operable to deliver recirculated fluid dropwise into said reservoir when said flow controller is in said first position.
- 35 14. An apparatus for detecting and enumerating a particulate analyte present in a liquid sample comprising at least one filter for retaining a particulate analyte,

a holder for supporting said filter, temperature control means for adjusting the filter to a selected temperature, a first conduit for delivering liquids to said filter and a second conduit for removing filtrates from said filter, 5 flow control means whereby said first and second conduits may be connected to form a closed loop for recirculation of fluid through the filter, a controller for controlling operation of said temperature control means and a pump for recirculation of fluid through the filter.

10

15. An apparatus according to claim 14 wherein said controller further controls operation of said pump.

16. A method for detecting and enumerating a 15 particulate analyte present in a liquid sample comprising delivering said liquid sample through an inlet duct to a filter element,

removing filtrate flowing through the filter element through an outlet duct while retaining the particulate 20 analyte on the filter element,

delivering a suitable reagent to said filter element while maintaining the filter element at a suitable temperature to fix a selected component of said particulate analyte to said filter element, and

25 delivering to the filter element suitable detection reagents to render the selected component detectable, whereupon, on delivery of said reagents, said inlet duct is connected directly or indirectly to said outlet duct

to permit recirculation of said reagents through said 30 filter element, and said filter element is subjected for a selected number of repetitions to a selected temperature cycle by operation of a heat transfer means operable to transfer heat to and from said filter element, said reagents being recirculated through said 35 filter element for selected time periods during said temperature cycle by operation of a pump, operation of said heat transfer means and said pump being controlled

by a controller whereby the particulate analyte is detected and enumerated.

17. A method in accordance with claim 16 wherein
5 said particulate analyte is a microorganism.

18. A method in accordance with claim 17 wherein
said microorganisms are bacteria.

10 19. A method in accordance with claim 17 where n
said selected component is a DNA sequence characteristic
of said microorganism, said detection reagents include
DNA polymerase and said selected component is rendered
detectable by amplification of said characteristic DNA
15 sequence.

20. An apparatus for detecting and enumerating a
particulate analyte present in a liquid sample comprising
at least one filter element supported by a holder,
20 at least one inlet duct communicating with said
filter element to deliver said liquid sample to said
filter element,

pump means connected to said inlet duct to provide
said liquid sample therethrough,

25 at least one outlet duct communicating with said
filter element to convey a filtered liquid sample
therefrom,

heat transfer means positioned adjacent to said filter
element for selectively transferring heat to and from said
30 filter element, and

control means connected to said heat transfer means
and said pump means, for controlling said heat transfer
means to maintain said filter element at preselected
temperatures for preselected time periods, and controlling
35 said pump means f r optionally recirculating a fluid
through said filter element, whereby said particulate
analyte is affixed to said filter element by said heat

transfer means to facilitate detection and enumeration of said particulate analyte present in said liquid sample.

21. An apparatus for detecting and enumerating a
5 particulate analyte present in a liquid sample comprising
at least one filter element supported by a holder for
retaining a particulate analyte,
temperature control means adjacent to said filter
element for adjusting said filter element to a selected
10 temperature,
a first conduit communicating with said filter element
for delivering a liquid sample to said filter element,
a second conduit communicating with said filter
element for removing a liquid sample from said filter
15 element,
flow control means connected to said first and second
conduits to form a closed loop for recirculation of fluid
therethrough, and
a controller means connected to said temperature
20 control means and said pump means, for controlling said
temperature control means and said pump means for
recirculation of a fluid through said filter element,
whereby said analyte is affixed to said filter element by
said temperature control means to facilitate detection and
25 enumeration of said particulate analyte present in said
liquid sample.

-
22. A method for detecting and enumerating a
particulate analyte present in a liquid sample comprising
30 delivering said liquid sample through an inlet duct to
a filter element,
removing filtrate flowing through the filter element
through an outlet duct while retaining the particulate
analyte on the filter element,
35 delivering a reagent to said filter element, while
maintaining the filter element at a suitable temperature,

to thereby fix a selected component of said particulate analyte to said filter element,

delivering to the filter element a detection reagent to render the selected component detectable following said
5 delivery of said reagent,

communicating a pump means to said inlet duct, said filter element and said outlet duct,

recirculating said reagent through said filter element, and subjecting said filter element to a selected
10 number of temperature cycles by operation of a heat transfer means operable to transfer heat to and from said filter element,

said reagent being recirculated through said filter element for selected time periods during said temperature
15 cycle by operation of said pump means, for collecting enumerable quantity of said particulate analyte, and enumerating said quantity of collected particulate analyte.

23. The method according to claim 22, wherein said
20 suitable temperature is about 45°C to about 94°C and said detection reagent is selected from the group consisting of Hoechst 3328 stain, DAPI, ethidium bromide, and a radiolabelled probe.



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FIG. 1.

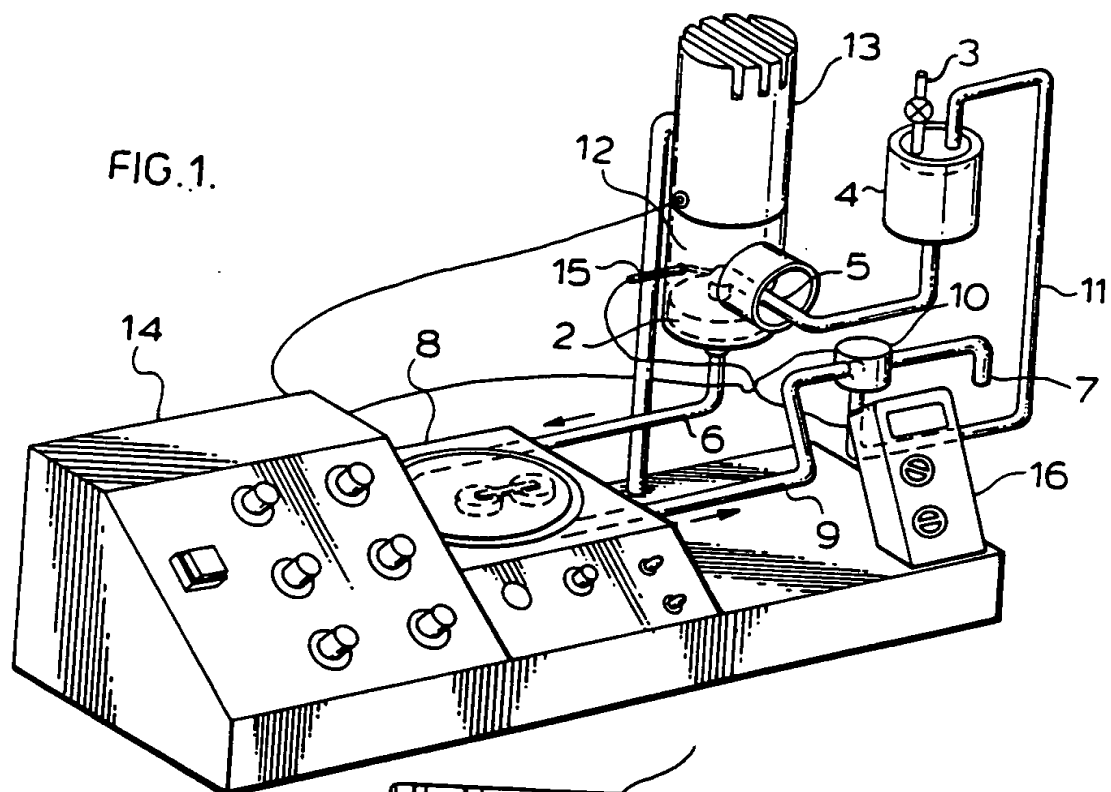
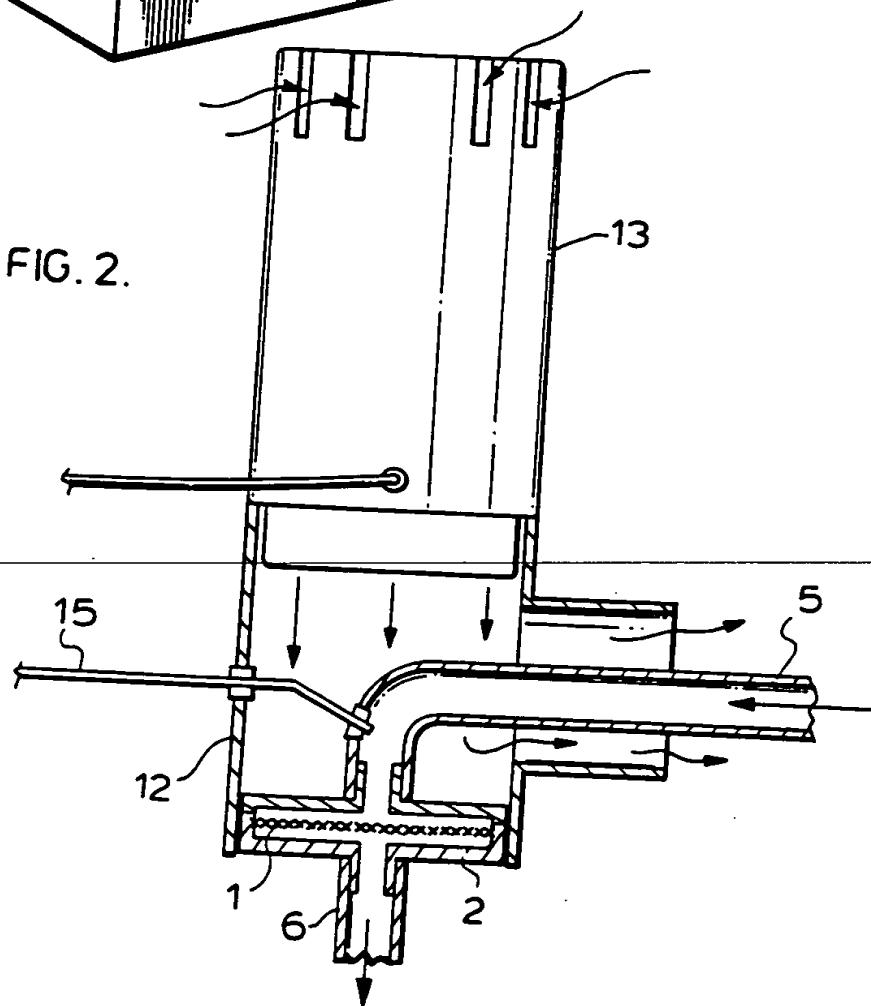


FIG. 2.





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FIG. 3.

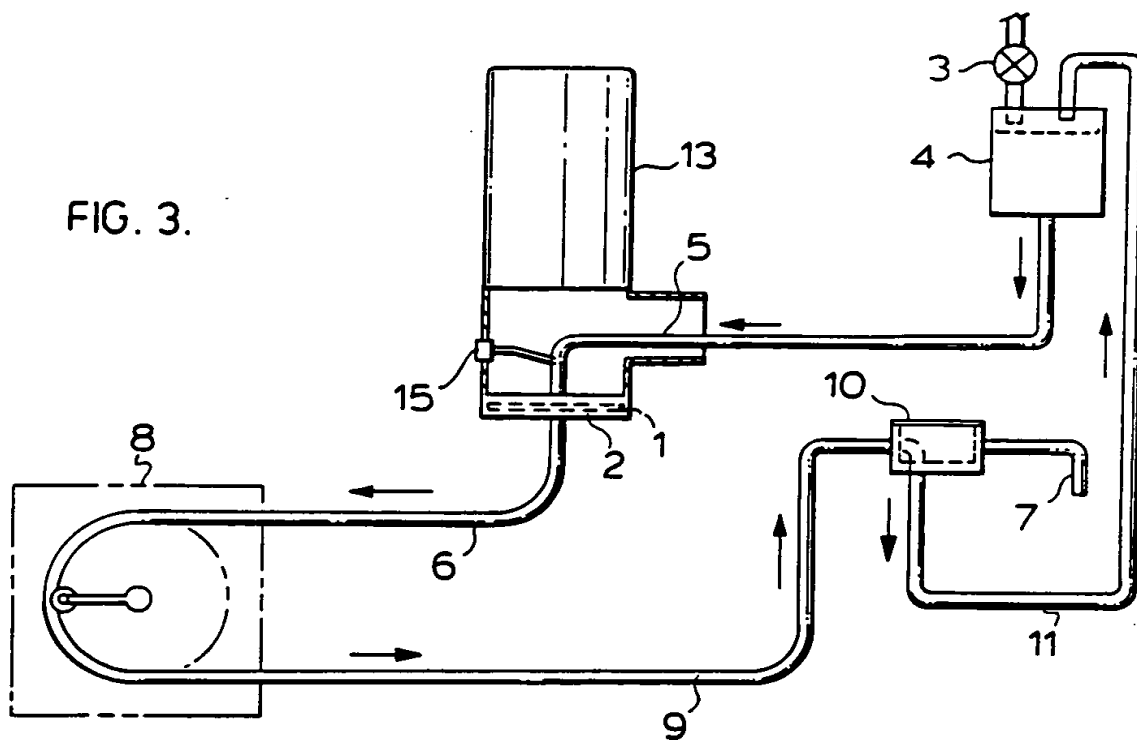
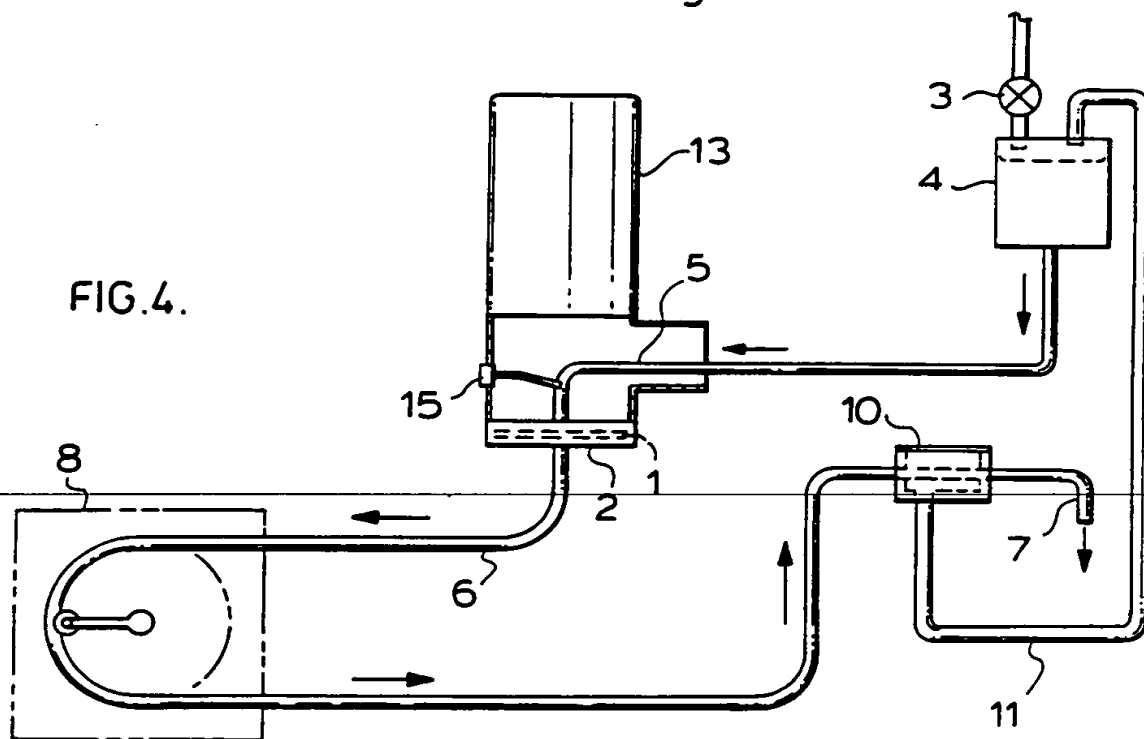


FIG. 4.





INTERNATIONAL SEARCH REPORT

Internat. Application No
PCT/CA 94/00101

A. CLASSIFICATION OF SUBJECT MATTER

C 12 M 1/12, C 12 Q 1/06, C 12 M 1/34, C 12 M 1/36,
C 12 M 1/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C 12 M, C 12 Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|------------------------|
| A | WO, A1, 91/06 670 (INFOMETRIX) 16 May 1991 (16.05.91), abstract. | 1, 16- 18, 20-22 |
| | -- | |
| A | US, A, 4 912 059 (NEWMAN et al.) 27 March 1990 (27.03.90), abstract. | 1, 20 |
| | ---- | |

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

05 May 1994

Date of mailing of the international search report

25. 05. 94

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ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
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PCT/CA 94/00101 SAE 85837

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| In Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche | Datum der Veröffentlichung Publication date Date de publication | Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets | Datum der Veröffentlichung Publication date Date de publication |
|--|--|--|--|
| | | | |
| WD A1 9106670 | 16-05-91 | EP A1 499624 | 26-08-92 |
| | | EP A4 499624 | 17-03-93 |
| | | JP T2 5500311 | 28-01-93 |
| | | US A 5254461 | 19-10-93 |
| US A 4912059 | 27-03-90 | keine - none - rien | |